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ECOLOGICALLY FRIENDLY BIO-REDUCTION OF GRAPHENE OXIDE BY STAPHYLOCOCCUS WARNERI

Zina Bakir AL-HILLI [1]

Al-Qasim Green University, Iraq

Abstract

This study demonstrates for the first time that the cell-free filtrate of Staphylococcus warneri bacteria can reduce Graphene Oxide (GO). A number of techniques were used to characterize the bio-reduced graphene oxide (BrGO); via the UV-Vis absorbency, scanning electron microscopy (SEM) images, X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR) analyses. The UV-Vis peak of absorption was around 270 nm. In SEM images, thin, wrinkled nanosheets layered on top of one another were seen. In the FTIR spectra, the distinct peaks relating to oxygen-containing functional groups diminished, whereas the hydroxyl, alkoxy, epoxy, and O-H deformation peaks totally vanished. According to XRD analysis, the peak of diffraction at 2θ= 24° corresponded to a d-spacing of around 0.36 nm. GO sheets have been effectively reduced using the S. warneri bacterium's cell-free filtrate. This method is ecologically friendly, excludes the poisonous reagents usage, cost-effective, besides offering an alternate-safe technique for a suitable graphene production to be applied in biomedical applications.

Keywords: Staphylococcus Warneri, Brgo, Graphene Nanosheets, XRD, SEM, FTIR, GO, Biomedical Applications.

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[1] zinaalhilli@vet.uoqasim.edu.iq, https://orcid.org/0000-0003-4296-5540

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Introduction

Graphene is a honeycomb-like structure made up of carbon atoms packed tightly together in two-dimensional sheets, and graphene compounds are biocompatible nanomaterials with exceptional properties [1,2]. By removing the oxygen-containing groups from GO, various chemical and physical procedures can be employed to rebuild the conjugated structure and generate reduced graphene oxide (rGO) [3,4]. Although, these technologies can manufacture complete graphene, their expensive costs and significant energy consumption prevent them from being widely used [5]. Chemical reduction procedures generate irreversible aggregations due to the Van der Waals attractive, strong forces between graphene planes, restricting their processability [6]. Additionally, the most commonly utilized chemical reduction agents, as with hydrazine and hydroquinone, are extremely harmful to both living organisms and the environment. In biological applications, the presence of miniscule quantities of these substances might be dangerous [7].

Biological procedures have been noted to be environmentally benign, widely available, affordable with a high production yield, devoid of poisonous chemicals, and easy to employ [8]. Bacterial systems have gained the interest of many researchers in latest years just as a potential alternative to chemical and physical strategies for reducing GO. Some bacterial species, such as *Shewanella oneidensis* [9], *Pseudomonas aeruginosa* [10], *Escherichia coli* [11], *Bacillus subtilis* [12], and *Desulfovibrio desulfuricans* [5], have all been shown to reduce GO safely and gently.

In this study, the cell-free filtrate of *S. warneri* bacteria was employed to test the potential for synthesizing rGO.

2. Methods of Experiment

2.1. Cell-free filtrate preparation

The procedure described by [13] was applied for preparing and activating bacterial growth.

2.2. Bio-reduction of GO nanosheets

Graphene oxide powder from Graphitene (UK) was utilized to begin the biosynthesis of rGO according to [14].

2.3. Characterization of BrGO

Using a Mega 2100 Double Beam Ultraviolet-Visible spectrophotometer (Scinco, Korea), at Babylon University, College of Pharmacy, UV-Vis spectroscopy of the watery dispersion of BrGO was performed. The morphology and thickness of BrGO- nanosheets was studied using scanning electron microscopy (SEM) (FEI NOVA NANOSEM 450I, Netherlands) at Babylon University, College of Pharmacy. The surface functional groups were investigated using Fourier transform infrared spectroscopy (FTIR) (Alpha-Bruker, Germany). At Al-Kufa University, Faculty of Science, Department of Chemistry. The spectrum was measured between 500 and 35,000 cm^{-1}. The BrGO powder's interlayer spacing was measured using X-ray diffraction (XRD). The sample was exposed to Cu-Ka (1.5418 A°) radiation at 40 kV and 40 mA using an XRD LAB 6000X diffractometer (SHIMADZU, Japan). XRD analysis was conducted at Baghdad University, College of Education for Pure Sciences (Ibn Al-Haitham), The Central Service Laboratory.

3. Results

The GO dispersion was stirred with the bacterial cell-free filtrate for 72 hours. As can be seen in Figure (1), the mixture changed from clear, brown to black in color with precipitates at the reduction reaction.

Figure 1: Photos of GO (left) and BrGO synthesized by *Staphylococcus warneri* (right)

The UV-Vis peak of BrGO in Figure (2) shows maximum absorption of around 270 nm.

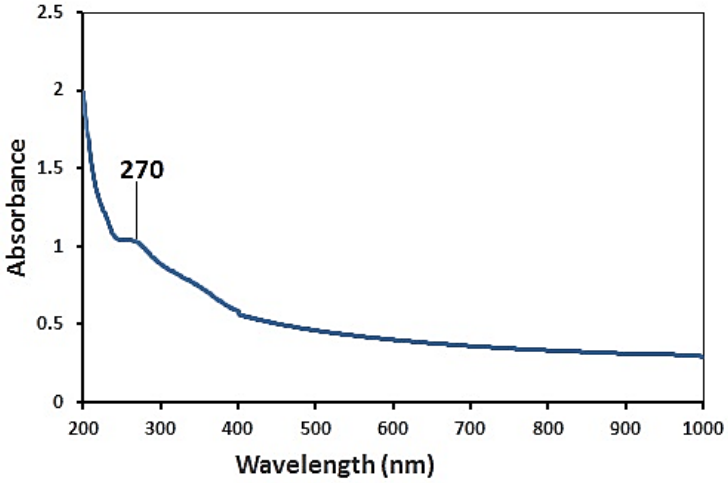

Figure 2: UV–Vis absorption spectrum of BrGO

In Figure (3), the SEM image of BrGO displays wrinkled, thin nanosheets piled on top of one another, with lateral dimensions ranging from a few micrometers in length to nearly 22 nm in thickness.

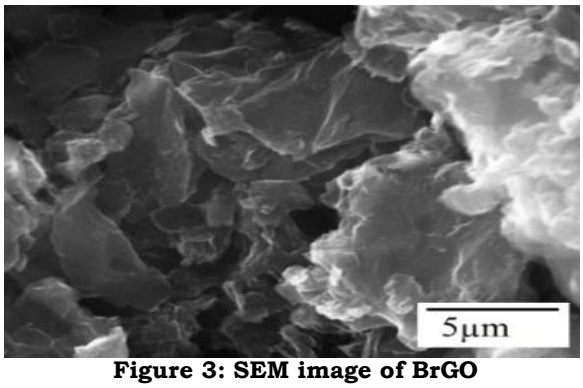

Figure 3: SEM image of BrGO

In the FTIR analysis, the distinctive peaks associated with oxygen-containing groups in BrGO-nanosheets diminished, whereas the hydroxyl, alkoxy, epoxy, and O-H deformation peaks totally vanished (Figure 4). Absorbance peaks were observed at 2325.32 and 2084.65 cm^{-1} (corresponding to C=O stretching vibrations), 1508.08 and 1652.40 cm^{-1} (C=C stretching vibrations from the aromatics).

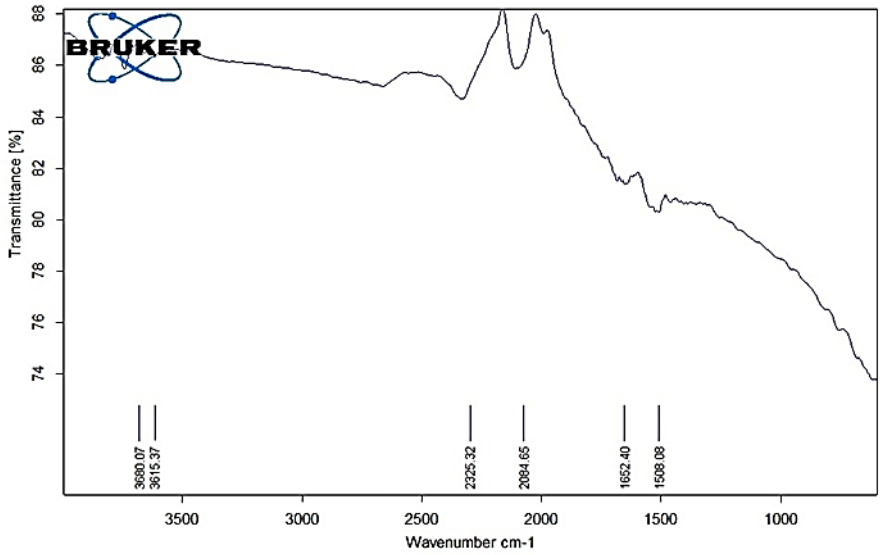

Figure 4: FTIR analysis of BrGO-nanosheets

The XRD-examination revealed that BrGO-sheets had a peak of diffraction at 2θ=24°, which corresponded to approximately 0.36 nm of layer spacing (Figure 5).

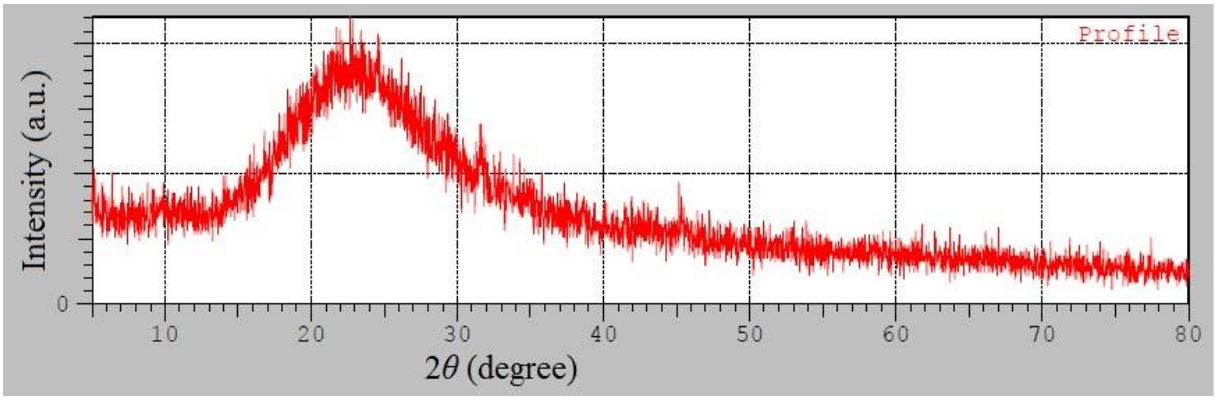

Figure 5: X-ray diffraction analysis of BrGO

This study demonstrates for the first time that a cell-free filtrate of *S. warneri* can reduce GO. This finding, as a result, expands the bacterial strains range capable of reducing GO beyond those previously documented.

4. Discussion:

The color of the mixture altered from clear, brown to black with precipitates throughout the reaction, indicating the bio-reduction process induced by the elimination of oxygen-containing bonds from GO [15,16]. The UV-Vis peak at 270 nm shows that GO has been successfully reduced by the bacterial filtrate and that electronic conjugation in BrGO-sheets has been recovered, allowing excitation of electrons at lower energies [17]. Equivalent UV-Vis absorption spectra were observed for rGO biosynthesized by β-carotene [18], and black tea leaf extract [19].

The SEM of BrGO is consistent with earlier investigations that found rGO nanosheets look like wrinkled, separated layers that build piled on top of each other by diverse self-assembly mechanisms [20,21]. The findings of FTIR spectra indicate deoxygenation of BrGO-sheets and restoration of π-conjugation [22], and are in accordance with those obtained using some bio-reducing agents [8,14,23].

The XRD examination points to the restacking of graphene sheets, ascribed to the elimination of most oxygen-containing groups amongst graphene layers, confirming the reduction of GO [24]. Several studies, on the other hand, found a broad peak in the 23-26° range [25, 26, 27].

5. Conclusion

Graphene oxide sheets have been successfully reduced using a cell-free filtrate of the *S. warneri* bacteria. Biosynthesis of graphene nanosheets was demonstrated by the UV-Vis absorbency, SEM images, XRD, and FTIR analyses. This approach is ecologically friendly, cost-effective, excludes the use of poisonous reagents, and offers an alternative safe technique for the production of graphene suitable for biomedical applications.

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